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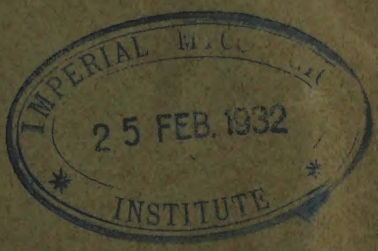

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A STUDY OF CERTAIN CHEMICAL TREATMENTS IN RELATION TO SEED-BORNE DISEASES OF CALAUAN YELLOW FLINT MAIZE[1]

VALERIANO C. CALMA, LORENZO G. PADERNA AND MACARIO A. PALO

Corn (*Zea mays* L.) is the second important crop in the Philippine Islands. In some provinces it constitutes the principal food of the people. In 1925 there were 552,380 hectares of land planted to corn in the Philippines and the crop was worth ₱30,767,250[2]. No survey of crop losses caused by the ravages of diseases has been made, but Baker (1917) and Reinking (1918) estimated them at about 10 per cent annually, which would mean a yearly loss of a few million pesos.

Seed-borne diseases are an important source of loss in growing corn. They either kill the seedlings or so weaken the plants that they easily succumb to the attack of other diseases. Weakened plants generally produce diseased ears which form sources of inocula for the neighboring plants and for the following crop.

The most practical method of controlling seed-borne disease is by careful seed selection while the corn is in the field. Holbert and Hoffer (1920) recommend that only matured ears of at least medium size from upright, sturdy stalks, or plants whose stalks and portions of the leaves are still green and whose ears are supported at a convenient height on strong and sound shanks should be selected for seed purposes. Ears that are sound may be singled out from among the diseased ones by the bright rich luster, plump, clean kernels, with well-developed germs, shallow and smooth indentation and decidedly horny endosperm. The shank attachment should be clean and free from any discoloration.

The study of the merits of poisonous compounds bearing certain trade names was made by treating the kernels of Calauan Yellow Flint maize harvested in the native way with the dust and solution of these substances. The soundest looking kernels of this corn often have incipient infections of seed-borne diseases. The work was conducted from October, 1924 to January, 1926 and from October, 1926 to February, 1928.

[1] Based on two theses presented for graduation with degree of Bachelor of Agriculture from the College of Agriculture by Valeriano C. Calma in 1926, and Lorenzo G. Paderna in 1928. This paper was written by Mr. Macario A. Palo. Theses and paper were prepared in Department of Plant Pathology under direction of Dr. G. O. Ocfemia.

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[2] One peso (₱1.00) = $0.50 U. S. currency.

REVIEW OF LITERATURE

Bolley (1897) states that stinking smut of wheat is controlled by immersing the grains in 1 to 1½: 1000 solution of bichloride of mercury and that oats soaked for two hours in 2½: 1000 formalin solution gave a higher percentage of germination and a greater yield than the untreated seeds.

Braun (1920) states that copper sulfate solution is ineffective and detrimental to germinating seeds.

Leukel, Dickson, and Johnson (1926) used 1.0, 0.5 and 0.25 per cent of aqueous solutions of Uspulun, Semesan, Germisan and Corona and found after seven years of extensive experiments that Germisan gave the best results, Uspulun the next best and Semesan the poorest.

Reddy, Holbert, and Erwin (1927) soaked Diplodia-infected corn kernels in 0.4 per cent solutions of Semesan and Uspulun and 0.25 per cent Corona 620 solution and found that Uspulun-soaked kernels gave the best field stand, Semesan-soaked the second best, Corona 620-soaked the third, and the controls, the poorest. These investigators found also that the kernels soaked in Uspulun gave higher yields than those soaked in Semesan and Corona 620.

Reddy and Holbert (1928) report that *Diplodia* seedling-blight of sweet corn can be controlled by dust or liquid treatment of the seed with any of the compounds including Semesan, Uspulun, Corona 640, Bayer dust and many others that they tested.

Holbert, Reddy, and Koehler (1928) found in their experiments on the chemical-dust seed treatments for dent corn that varieties differ in the degree to which they are benefited by those treatments and that they are not always followed by an increase in yield. These authors used Bayer dust, Improved Semesan Jr., and Merko.

MATERIALS AND METHODS

Seed

Ears of Calauan Yellow Flint maize were obtained from the Department of Agronomy. The kernels two centimeters from the butt and five centimeters from the tip of the ears were discarded so that only large and fairly uniform kernels were used.

Chemicals

Copper sulfate, sulfur powder, formaldehyde, Derris powder, mercuric chloride and organic mercury compounds known under the trade names of Germisan[3], Uspulun[4], Semesan[5], Corona 640[6], Corona 640-S[7], and Colloidal copper[8] were used.

[3] Obtained from Saccharin-Fabrik in Germany.

[4] Obtained from Bayer Company of New York.

[5] Obtained from E. I. Du Pont de Nemours Co., Inc., Wilmington, Delaware.

[6], [7], [8] Obtained from Pittsburgh Plate Glass Company, Corona Chemical Division, Milwaukee, Wisconsin.

Formalin and mercuric chloride were used in the form of solutions which were prepared in concentrations of 0.1 per cent and 0.2 per cent. Germisan, Semesan, Uspulun, Colloidal copper, copper sulfate, Corona 640, and Corona 640-S were prepared in concentrations of 0.25 per cent, 0.50 per cent, 0.75 per cent and 1.0 per cent. Germisan, Uspulun, Corona 640, and Corona 640-S were used also in the form of dust with Bayer's Dipdust and Special dust and powder of *Derris elliptica*, *Derris polyantha*, and *Derris philippinensis*.

Determination of the seed-borne flora of Yellow Flint maize and the tissues wherein the organisms are carried

The seed-borne flora of the maize was determined by plating out the entire kernels of corn, kernels without seed coat, endosperms alone, and embryos alone on potato-dextrose agar, agar in water, and nutrient agar in petri dishes. The results obtained are summarized in table 1.

TABLE 1

The fungous flora of Calauan Yellow Flint maize

ORGANISMS OBTAINED[a]	PERCENTAGE INFECTION OF PARTS PLATED OUT ON POTATO-DEXTROSE AGAR, WATER AGAR, AND NUTRIENT AGAR.			
	Entire kernels	Kernels without seed coat	Endosperm alone	Embryo alone
Fusarium	100	80	80	70
Black mold	100	70	70	70
Yellow mold	40	20	10	30
Blue mold	20	10	20	10

[a] No effort was made to determine the identity of the *Fusarium* and molds obtained.

It may be seen in table 1 that *Fusarium* sp. and black mold are the predominating fungous flora in the seeds of Calauan Yellow Flint maize.

Tests of the efficiency of the chemicals used

The corn kernels were immersed in the solutions of Germisan, Uspulun, Semesan, Formalin, mercuric chloride, copper sulfate, Corona 640, Corona 640-S and Colloidal copper in test tubes for varying lengths of time covering the kernels to a depth of at least one centimeter. Every treatment had a control in which the kernels were soaked for the same length of time in sterile water. After the treatment, the corn kernels, including those in the checks, were spread on large filter papers to air-dry. Then the kernels were planted in sterilized soil in boxes. The plants were observed daily to determine the date of emergence, the percentage of germination, and condition of seedlings.

In the dust treatments the kernels were moistened with sterile water and then rolled until they were thoroughly covered with the fine powder of Germisan, Uspulun, Semesan, Dipdust, Special dust, Corona 640, Corona 640-S, powder of *Derris elliptica*, *Derris polyantha* and *Derris philippinensis*. The kernels used as control were moistened with sterile water as described by Leukel, Dickson, and Johnson (1926).

To determine the effect of the treatment on the production of ears, tests were conducted in the field. Only the concentrations and the lengths of immersion from the laboratory tests which indicated beneficial effects upon the seedlings and effectiveness in the control of seed-borne organisms were used.

The solutions used in field tests and their concentrations and lengths of immersion are given in table 2.

The dusts were not used in field tests because the seedlings from the treated kernels were the same as those of the control, and in some instances the treated seeds were poorer than those of the control. This result is in accord with the experience of Leukel, Dickson, and Johnson (1926) but contrary to that of Reddy and Holbert (1928) who reported that dust seed treatments for the control of *Diplodia* seedling blight of sweet corn are as beneficial as the liquid treatments and have the additional advantage of being more easily applied and involving less injury to the seed.

RESULTS

Laboratory experiments

In the laboratory experiments, soil sterilized with heat and placed in seed boxes was used. Previous to the tests with the chemicals, experiments carried on to determine whether or not soil sterilization has deleterious effects upon corn seedlings showed that it produces beneficial effects.

The criteria used for measuring the effects upon the corn of the various treatments in laboratory tests were: (1) height of the seedlings from fixed points on the surface of the soil to the tips of the longest leaf; (2) size of the shoots; (3) extent of the root systems; and (4) color of the vegetative parts as compared with those from untreated kernels.

Of the different chemical solutions used in the laboratory experiments, Germisan gave the best stand of seedlings. It was followed in success by formalin, next by Uspulun, then by Semesan. Mercuric chloride solution gave the poorest result.

Field experiments

Wet and dry season plantings were carried on in the experimental plot of the Department of Plant Pathology. The chemical solutions which showed controlling effects of seed-borne organisms and produced stimulatory effects upon the seedlings, and the lengths of immersion in these solutions

which gave the best results in the laboratory tests were tried out in the field.

Unfortunately, the soil of the plot that was assigned to the field trials was very poor because it had been planted to sugar cane, rice, tobacco, and corn repeatedly. The land was thoroughly prepared and furrows run 75 centimeters apart. During the wet season only one corn kernel was planted in each of the hills which were 70 centimeters apart in the furrows. In the dry season planting two kernels were planted in hills which were 75 centimeters apart. In both wet-and dry-season trials the treated corn kernels and the checks were planted in alternate furrows. The percentage of emergence in the wet-and dry-season tests did not agree with the percentage of germination in the laboratory tests. It was found that in the wet season plantings there was a higher percentage of emergence than in the dry season plantings.

When the plants were about 30 centimeters tall, leaf spots caused by *Ophiobolus heterostrophus* Drechsler and leaf blight caused by *Helminthosporium inconspicuum* Ell. and Evht. were present on the leaves of the plants. There were more leaf diseases in the wet season crop than in the dry season planting.

The tasseling of the corn of the wet-and dry-season plantings was not affected by the treatments. Both the control and the plants from treated kernels tasseled at about the same time.

The number and weight of the ears from each row and the number of diseased ears and nubbins were noted. The results are given in table 2.

The results obtained from the wet and dry season plantings of treated kernels may be summarized as follows:

1. None of the treated kernels in the wet and dry season plantings in the field gave 100 per cent germination. In some cases there were control kernels that gave a higher percentage of emergence than the treated kernels.
2. The wet season planting gave higher percentages of germination than the dry season planting.
3. All plants from treated kernels gave higher yields of ears than those of the control kernels.
4. The wet season planting produced more and larger ears than the dry season planting.
5. There were more diseased ears in the wet season planting than in the dry season crop.

As shown in table 2 the treatment that gave the highest yield with the use of formalin, mercuric chloride, Semesan, and Uspulun in the wet season planting did not give correspondingly the highest yield in the dry season planting. The kernels that were soaked in 0.25 per cent solution of Germisan for 8½ hours, however, gave the best yield in both wet and dry season plantings. By close inspection of the data in table 2 it may be seen that the formalin treatments that may be recommended to use for the control

of seed-borne diseases of Calauan Yellow Flint maize is immersion of the seeds for 7 hours in 0.1 per cent and 5½ hours in 0.2 per cent solution of this chemical. With mercuric chloride the treatments that gave beneficial results were .01 per cent for 5 hours and 0.02 per cent for 4½ hours; with Semesan 0.50 per cent and 0.75 per cent for 2½ to 3½ hours; and with Uspulun 0.5 per cent for 4 to 4½ hours and 0.75 per cent for 3½ hours.

Of the liquid treatments the results of which are shown in table 2, Germisan proved to be the best of all. Uspulun was second best and Semesan came third. The beneficial effects of these three chemicals upon corn agree with those reported by Leukel, Dickson, and Johnson (1926). Mercuric chloride and formalin, though slightly beneficial in a way, gave poorer results than either Germisan, Uspulun or Semesan. The average yield obtained from the treatment with formalin and with mercuric chloride was almost the same.

Table 2 shows also that none of the seed treatments completely rid the subsequent crop of disease in the ears. Diseased ears were obtained from both wet and dry season plantings with the exception of Uspulun-soaked seeds which did not produce any diseased ear in the dry season planting. Fewer diseased ears were obtained from the Germisan seed treatment than from any of the other chemical treatments given in table 2. The greatest number of diseased ears was obtained from mercuric chloride seed treatment.

DISCUSSION OF RESULTS

In this work it was noted that the kernels of Calauan Yellow Flint maize, although they appear healthy, may carry *Fusarium* and black, yellow, and blue molds on or within the kernels. The kernels treated with weak aqueous solutions of Germisan, formalin, Uspulun, Semesan and mercuric chloride gave a higher percentage of germination and better growth of seedlings than the control. Reddy and Holbert (1928) state that the roots of sweet corn grow rapidly into the soil and the aërial parts develop early when the treatment controlled the disease without any injury. These authors used Uspulun, Semesan, Corona 640, and other chemical dusts. Tisdale, Taylor, and Griffiths (1925) found that aqueous solutions of Germisan, Semesan, and Uspulun give excellent results in the control of barley smut. In these authors' experiments, Germisan, Uspulun, Semesan, Dipdust, Special dust, Corona 640, and Corona 640-S dusts were found to be unsatisfactory in the control of smuts of oats and barley. Leukel, Dickson, and Johnson (1926) found that dust treatment failed to give any satisfactory results in the control of barley stripe. Calma and Paderna also found that chemical dust treatment for the control of *Fusarium* and molds in the seed of Calauan Yellow Flint corn was of no avail. Of the chemicals used in solution in the laboratory and field tests they noted that Germisan was the best substance for seed treatment of corn. They found that formalin treatment was next to Germisan in laboratory tests but in field tests Uspulun was much better than formalin in the production of ears.

The results obtained by Calma and Paderna during wet and dry season plantings are in accord with those of Leukel, Dickson, and Johnson (1926). Their results also agree with those of Reddy, Holbert, and Erwin (1927) who state that sweet corn kernels treated with Uspulun solution gave a higher yield of ears than those treated with Semesan and Corona 640.

In wet and dry season plantings, Calma and Paderna noted that there was no difference in the time of emergence of seedlings between the treated and the control plants. All corn kernels in the treated and control rows emerged after three days from the time of planting. Plants from treated kernels during wet and dry season plantings were of course more vigorous and gave a better stand than those of the controls.

The treated kernels planted during the dry season produced ears that were much less infected than the treated kernels planted during the wet season. The average size of ears produced from the treated seeds during the wet and dry season crops was larger than the average size of ears produced in the control. In the wet season crop the average size of ears from the treated seeds were larger than the average size of ears from the treated seeds in the dry season crop. The ears produced from treated kernels planted during the dry season were poorer than those produced during the wet season. The difference in production between the treated seeds and the control was very marked in both the wet and dry season plantings.

The use of these organic mercury compounds is prohibitive under our present farming conditions in the Philippine Islands. As they are deadly poisonous to man and animals extreme care should be taken when handling them. However, the writers believe that, at least for technical purposes, Germisan, Uspulun and Semesan merit further tests not only with maize but with other seeds.

SUMMARY

1. *Fusarium*, black, yellow, and blue molds are the seed-borne fungi in the kernels of Calauan Yellow Flint maize.

2. The fungi are carried on the seed coat, and in the endosperm and embryo of the kernels.

3. Treatment of seeds with chemicals in the form of aqueous solutions produced beneficial effects on the seedlings in the wet and dry season plantings and controlled the seed-borne diseases. The treatments did not affect the time of emergence and the tasseling of corn.

4. Of the five chemicals used for treating the kernels for the wet and dry season plantings, Germisan gave the best results in the production of ears. Uspulun ranked second as to production of ears and control of seed-borne diseases. Semesan ranked third, and formalin, fourth. Mercuric chloride solution gave the poorest results of the chemicals used, although treated kernels produced a yield higher than the control and it reduced the amount of injury caused by seed-borne diseases.

5. More disease-free ears were produced from kernels treated with the five chemical solutions than from the control.

6. Aqueous solutions of Germisan, Uspulun, and Semesan merit further tests for seed treatment in technical ways not only with maize but with other grains or seeds. As these preparations are deadly poison to man and animals and are also expensive they will not become popular under Philippine conditions.

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TABLE 2

The effect of seed treatment upon the yield of Calauan Yellow Flint maize during the wet (W) and dry (D) seasons of 1927–1928

CHEMICALS	STRENGTH OF SOLUTION	LENGTH OF IMMERSION IN SOLUTION	NUMBER OF KERNELS PLANTED	WEIGHT OF FIVE SEEDLINGS ON AIR DRY BASIS	NUMBER OF STERILE EARS		NUMBER OF DISEASED EARS		TOTAL NUMBER OF EARS		TOTAL WEIGHT OF EARS		AVERAGE WEIGHT OF EARS		AVERAGE WEIGHT OF EARS OF ALL TREATMENTS			
					W	D	W	D	W	D	W	D	W	D	W		D	
															Treated	Control	Treated	Control
	per cent	*hours*		*grams*							*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Formalin	0.1	3½	10	3.5	0	0	1	1	7	6	625.0	272.0	89.28	45.33				
Control		3½	10	1.1	1	0	3	1	7	6	400.0	190.2	57.14	31.70				
Formalin	0.1	4½	10	2.4	0	0	1	0	8	6	825.7	320.4	103.21	53.40				
Control		4½	10	1.9	0	0	5	3	8	6	550.0	158.2	68.75	26.36				
Formalin	0.1	5	10	3.0	1	1	1	0	10	6	910.0	156.2	91.00	26.03				
Control		5	10	1.3	0	1	1	3	10	6	650.0	118.0	65.20	19.66				
Formalin	0.1	5½	10	3.1	0	0	2	1	9	7	713.3	258.9	79.25	36.98				
Control		5½	10	1.2	1	1	4	2	9	7	525.3	115.7	58.36	16.52				
Formalin	0.1	7	10	3.5	0	0	1	0	8	6	900.0	168.0	112.50	28.00				
Control		7	10	1.78	0	1	4	3	8	6	575.0	101.2	71.87	16.86				
Formalin	0.2	5½	10	3.6	0	0	0	1	9	7	925.4	336.6	102.82	48.08				
Control		5½	10	1.5	2	1	4	2	9	7	650.0	146.2	72.22	20.88				
Formalin	0.2	6	10	2.5	2	0	0	0	9	7	950.0	321.3	105.55	45.90				
Control		6	10	1.9	2	2	4	1	10	9	600.0	398.1	60.00	44.23	97.66	64.77	40.53	25.17
Germisan	0.25	8	10	4.1	0	0	1	0	9	7	1125.0	494.9	125.00	70.70				
Control		8	10	0.89	1	1	6	2	10	7	600.0	171.3	60.00	24.47				
Germisan	0.25	8½	10	3.8	1	0	0	0	10	9	1450.4	872.0	154.04	96.88				
Control		8½	10	1.2	0	2	6	1	7	7	750.0	217.1	107.14	31.01				
Germisan	0.50	5½	10	3.9	0	0	0	0	8	9	1050.4	699.4	131.30	77.71				
Control		5½	10	1.8	1	0	4	4	7	9	450.0	296.3	64.29	32.92				
Germisan	0.50	7½	10	4.0	0	0	0	0	10	9	1250.0	537.4	125.00	59.71				
Control		7½	10	1.6	0	0	5	3	8	8	550.0	259.4	68.75	32.42				
Germisan	0.50	8	10	3.7	0	0	1	0	9	10	1100.0	674.2	122.22	67.42				
Control		8	10	1.7	1	0	4	1	8	6	600.0	251.5	75.00	41.91				
Germisan	0.75	5½	10	3.5	0	0	0	4	9	8	1250.0	457.8	138.88	57.22				
Control		5½	10	1.94	1	3	7	4	8	8	800.9	145.7	100.11	18.21				
Germisan	0.75	6	10	2.9	0	0	0	0	9	9	1025.0	648.2	113.88	72.02				
Control		6	10	1.8	1	2	3	4	7	9	450.0	272.8	64.28	30.31				
Germisan	1.00	3	10	2.4	0	0	1	0	9	8	1000.0	693.0	111.11	86.62				
Control		3	10	1.5	1	0	3	3	7	8	475.0	305.0	67.85	38.12				
Cermisan	1.00	4	10	2.7	0	0	1	0	9	9	925.0	723.6	102.77	80.40				
Control		4	10	1.9	1	0	3	3	7	9	350.0	337.9	50.00	37.54	123.91	73.04	74.29	31.88
Mercuric chloride	0.01	4	10	2.2	0	0	2	1	9	5	1025.0	102.8	113.88	20.56				
Control		4	10	1.2	1	3	3	0	7	3	350.0	31.6	50.00	10.53				
Mercuric chloride	0.01	5	10	2.4	0	0	2	1	8	6	800.8	254.2	100.10	42.36				
Control		5	10	1.98	1	0	4	1	8	6	575.0	106.6	71.87	17.76				
Mercuric chloride	0.02	3½	10	2.2	0	0	2	0	7	9	550.3	322.6	78.61	35.84				
Control		3½	10	1.3	1	1	3	3	7	8	440.5	73.8	62.92	9.22				
Mercuric chloride	0.02	4½	10	2.6	0	1	2	0	8	6	850.0	332.9	106.25	55.48				
Control		4½	10	1.2	0	0	3	1	7	6	413.0	106.5	59.00	17.75	99.71	60.94	38.56	13.81
Semesan	0.25	8	10	2.7	1	0	1	0	8	7	700.1	191.3	87.51	27.32				
Control		8	10	1.7	3	2	4	2	9	7	450.0	66.0	50.00	9.42				
Semesan	0.50	2½	10	3.2	0	1	1	0	8	7	800.5	374.4	100.06	53.48				
Control		2½	10	1.8	1	2	1	1	8	6	550.0	144.9	68.75	24.15				
Semesan	0.75	3½	10	2.8	0	0	1	1	8	7	825.0	372.0	103.12	53.14				
Control		3½	10	1.56	2	1	7	2	8	7	525.5	170.9	65.68	24.41				
Semesan	0.75	4	10	3.3	0	0	0	0	8	6	950.0	159.2	118.75	26.53				
Control		4	10	1.75	2	1	1	3	9	8	150.0	79.9	16.66	9.98				
Semesan	1.00	2½	10	2.4	0	1	2	0	8	5	900.0	177.2	112.50	35.44				
Control		2½	10	1.9	0	1	4	3	8	6	620.0	90.4	77.50	15.07	104.38	55.71	39.18	16.60
Uspulun	0.25	8	10	3.3	1	0	1	0	9	6	788.1	352.5	87.56	58.75				
Control		8	10	1.5	3	2	4	1	8	6	575.0	145.2	71.87	24.20				
Uspulun	0.50	4	10	2.8	0	0	2	0	8	8	910.9	379.1	113.86	47.38				
Control		4	10	1.4	0	2	4	2	8	6	789.5	190.1	98.68	31.68				
Uspulun	0.50	4½	10	3.2	0	0	1	0	9	6	900.0	546.1	100.00	91.02				
Control		4½	10	1.34	0	1	4	1	8	6	600.0	158.1	75.00	26.35				
Uspulun	0.75	3½	10	2.9	0	1	0	0	9	8	1250.2	313.8	138.90	39.22				
Control		3½	10	1.46	2	0	3	3	9	7	750.3	139.2	83.36	19.89				
Uspulun	0.75	4½	10	2.5	2	0	1	0	9	7	1000.0	591.9	111.11	84.55				
Control		4½	10	1.2	0	0	4	1	8	7	600.0	220.9	75.00	31.55				
Uspulun	1.00	2	10	2.9	1	0	1	0	9	6	950.0	546.6	105.55	91.10				
Control		2	10	1.23	1	1	3	2	8	7	750.0	112.4	93.75	16.06				
Uspulun	1.00	2½	10	4.0	0	0	0	0	10	6	1025.0	350.4	102.50	58.40				
Control		2½	10	1.8	4	2	0	2	9	7	600.8	230.8	66.75	32.97	108.49	80.57	67.20	26.10